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APOLIPOPROTEIN A-II AND SERUM HIGH-DENSITY LIPOPROTEINS

A Model System for the Study of Protein-Lipid Interactions at a Natural Hydrophilic-Hydrophobic Interface

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The precise role played by apolipoproteins in the overall structural organization of plasma lipoproteins is still unknown (1). The A apolipoproteins are the principal constituents of the high density lipoprotein (HDL) class. In man and nonhuman primates HDL contains both apo A-I and apo A-II. Human apo A-II consists of two identical chains linked by a single disulfide bridge, whereas in the monkey, apo A-II is a single chain molecule due to the absence of cisteine. The only disulfide of human apo A-II is readily amenable to reduction and alkylation, thus permitting the in vitro production of human single-chain apo A-II. In lower animal species, HDL contains no apo A-II; yet this lipoprotein has structural features which closely resemble those of man.

RESULTS

Based on the above information and on the fact that the functional role of apo A-II is yet undetermined, we elected to use canine HDL as a model to study the structural and functional effects caused on this lipoprotein by the addition of graded amounts of two- or one-chain apo A-II. These studies were favored by the knowledge of the properties of apo A-I and apo A-II in solution showing a different mode of self-association for apo A-I and apo A-II and between two-chain or one-chain apo A-II, whether naturally occurring (rhesus monkey) or obtained from the reduction and alkylation of human apo A-II (2). We also have evidence for a greater affinity of apo A-II relative to apo A-I for the hydrophilic:hydrophobic interface. At the glass bead:water interface, it has been estimated[1] that the ΔG_{aff} for apo A-I is -9.1 kcal/mol and that of apo A-II is -10.5 kcal/mol.

The addition at room temperature of lipid-free human apo A-II (two-chain) to canine HDL results in the uptake of this apoprotein by HDL and a concomitant displacement of apo A-I into the aqueous solution (3). The reaction, which is attended by no loss of lipid, is stoichiometric in that by varying the initial free apo A-II-apo A-I (HDL) ratio, one can show that per mole of apo A-I displaced, there are 2 mol apo A-II occupying the HDL surface. Thus, one obtains hybrid particles having the lipids of canine HDL and varying proportions of human apo A-II and canine apo A-I at the surface. In the extreme case, all of the protein mass in the hybrid particle is apo A-II with a calculated maximal occupancy of 6 mol/particle (~ 220,000 mol wt). In such a system apo A-II is never found free in solution even in the presence of phospholipid vesicles, which act as scavengers of the released apo A-I.

To a lesser degree, single-chain apo A-II either from natural sources (rhesus apo A-II) or reduced and alkylated human apo A-II are also effective (4) in displacing apo A-I from the surface of canine HDL, and the hybrid products formed exhibit differences which are both quantitative and qualitative. As shown in Fig. 1, the end products of the interaction between single-chain apo A-II and canine HDL are the HDL-apo A-II hybrid and free apo A-I. In turn, the incubation of two-chain apo A-II with canine HDL results in an additional component likely representing an apo A-I:apo A-II complex containing ~ 7% phospholipids. This component is particularly evident when two-chain apo A-II is incubated with HDL in apo A-II:apo A-I molar ratios exceeding maximal apoprotein

[1]Shen, B. Unpublished results.

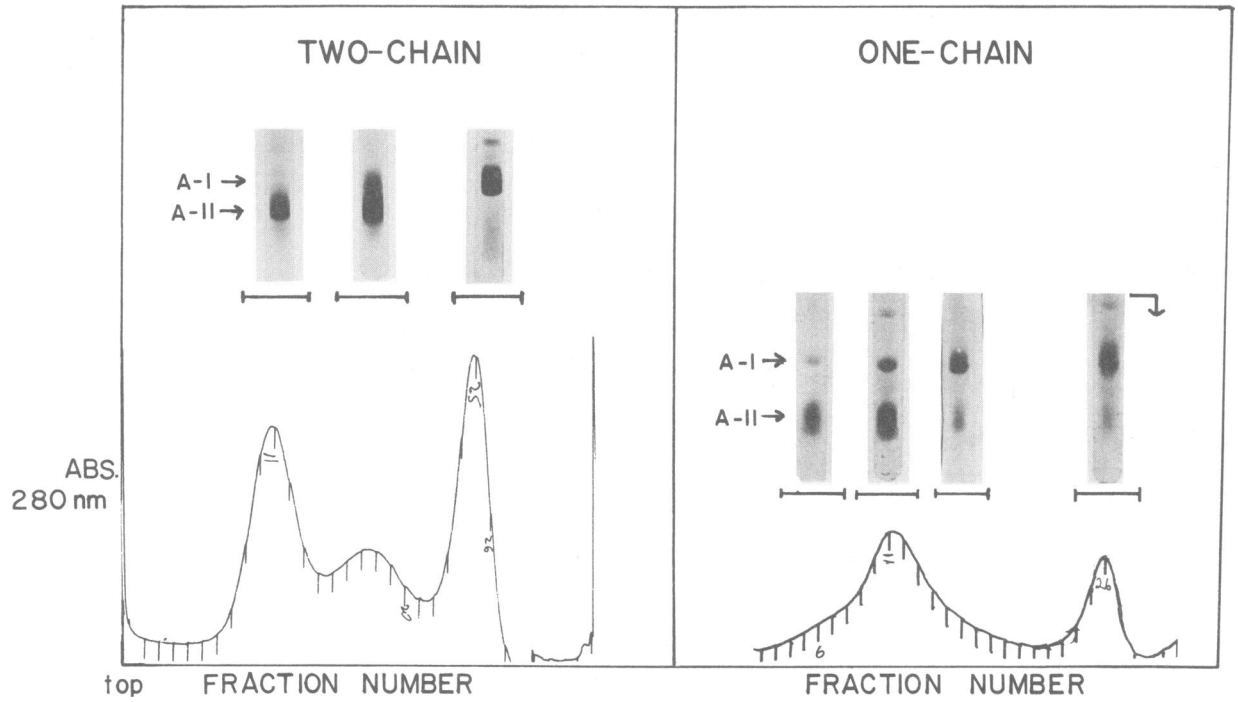

FIGURE 1 Density gradient elution profiles and SDS gel electrophoretic patterns obtained for products formed upon interaction of two- and one-chain apo A-II with canine HDL. Apo A-II either two-chain or *S*-carboxymethylated one-chain (reduced with mercaptoethanol and alkylated with iodoacetamide) was incubated at 25°C for 30 min with canine HDL at an initial A-II:A-I weight ratio of 3.5:1. The mixture was then centrifuged on a curvilinear NaBr density gradient (d 1.075–1.35 g/ml) in a Beckman SW 40 rotor at 14°C for 66 h (Beckman Instruments, Fullerton, CA). The eluates were simultaneously scanned at 280 nm and collected (0.4 ml/fraction). Electrophoresis of each fraction was carried out in 0.1% SDS-10% acrylamide and subsequently stained with Coomassie Blue.

occupancy on the lipoprotein surface. We have also observed this component in experiments wherein hybrids containing apo A-II and apo A-I in a weight ratio of 1:1 were cross-linked with dimethylsuberimidate and then subjected to the displacing action of two-chain apo A-II. The main resulting product was the cross-linked HDL hybrid resistant to the action of apo A-II, and an added component banding at d 1.15 g/ml similar in composition to the apo A-I:apo A-II:phospholipid complex noted previously.

DISCUSSION

Several points emerge from these results: (*a*) apo A-II prefers the hydrophilic-hydrophobic interface of the HDL particle over the aqueous environment; (*b*) apo A-II has a significantly greater affinity than apo A-I for the HDL surface; (*c*) this difference in affinity appears to be responsible for the displacement of apo A-I by apo A-II from the HDL surface; (*d*) contrary to apo A-II, apo A-I can be free in the aqueous medium likely to be in equilibrium with the molecules at the HDL surface; (*e*) the overall structural organization of HDL is unaffected whether apo A-I or apo A-II alone or mixtures thereof are at the HDL surface.

The implication of these findings in terms of HDL structure is that apo A-I does not interact strongly with any component of the lipoprotein particle and that its conformation at the interface permits this apoprotein to be replaced by an adequate amphiphile such as apo A-II. The latter has a comparatively stronger affinity for the HDL surface and thus can readily occupy areas previously containing apo A-I.

These conclusions may not be limited to the HDL particles. Studies in this laboratory have shown that a similar competition of apo A-II and apo A-I for the hydrophilic-hydrophobic interface occurs when using single-bilayer vesicles with a phosphatidylcholine:cholesterol molar ratio of 4:1 (5). A possible physiological significance of these findings comes from the observation that the modulation of the proportions of apo A-II and apo A-I in HDL is one of the determinants in the action of the enzyme lecithin:cholesterol acyl transferase. These studies have shown that apo A-II can inhibit the action of this enzyme by displacing activator apo A-I from the HDL particle.

Received for publication 5 May 1981.

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